Biosignature Analysis of Mars Soil Analogues from the Atacama desert: Challenges and Implications for future Missions to Mars

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Abstract

The detection of biosignatures on Mars is of outstanding interest in current Astrobiology and drives various fields of research, ranging from new sample collection strategies to the development of more sensitive detection techniques. Detailed analysis of the organic content in Mars analogue materials collected from extreme environments on Earth improves the current understanding of biosignature preservation and detection under conditions similar to Mars. In this paper we examined the biological fingerprint of several locations in the Atacama desert (Chile) which include different wet and dry, and intermediate to high elevation salt flats (also named salars). Liquid Chromatography and Multidimensional Gas Chromatography Mass Spectrometry measurement techniques were used for the detection and analysis of amino acids extracted from the salt crusts and sediments using sophisticated extraction procedures. Illumina 16S amplicon sequencing was used for the identification of microbial communities associated with the different sample locations. Although amino acid load and organic carbon and nitrogen quantities were generally low, it was found that most of the samples harbored complex and versatile microbial communities, which were dominated by (extremely) halophilic microorganisms (most notably by species of the Archaeal family *Halobacteriaceae*). The dominance of salts (i.e. halites and sulphates) in the investigated samples leaves its mark on the composition of the microbial communities but does not appear to hinder the potential of life to flourish since it

can clearly adapt to the higher concentrations. Although the Atacama desert is one of the driest and harshest environments on Earth, it is shown that there are still sub-locations where life is able to maintain a foothold, and as such, salt flats could be considered as interesting targets for future life exploration missions on Mars.

Keywords (3-6): Mars, Atacama Desert, Biosignatures, Amino Acids, Microbial Communities, Organic Carbon

1 Introduction

Gaining more insight in the geological, as well as the potential biological history of Mars is still a major outstanding goal for the planetary community. Although the past few decades have supplied us with large amounts of scientific data concerning mineralogy, geological record, current and past environmental conditions (including those that could support life), the presence of water and even of organic molecules on Mars, more questions remain. Many of these questions can only be directly addressed by *in situ* research on the red planet itself. However, Mars exploration missions are expensive, complex and time consuming. Therefore the amount and type of research that can be done on the red planet itself is still limited. However, certain terrestrial environments can serve as analogues for selected, past Martian environments. One of the most stringent questions is of course whether there is, or once has been life on Mars. This leads to the follow-up questions such as: How to look for signs of (extinct) life? Which type of biosignatures are appropriate targets (stability vs diagnostic power)? Where would chances be highest of finding such biomarkers? In order to increase the chances for successful detection, it is important to assess these questions to our best ability. In this regard, much information can be acquired by investigating terrestrial Mars analogue environments.

One of the most famous and well-studied Mars analogue environments is the Atacama desert in Chile. The Atacama desert is known as the driest, non-polar desert on Earth, receiving only a few millimetres of rain per year (Clarke, 2006). The region in the northern part of Chile and the Central Andes, which encompasses the Atacama desert, contains a multitude of north to south trending mountain ranges and closed basins, of which the central parts are often filled with evaporate deposits or, in more rare cases, highly saline waters (collectively known as salars) (Figure 1). Due to the desiccated conditions and lack of significant weathering effects, salts have accumulated in the region over the course of millions of years, resulting in the presence of an overall high salinity in the local soils (Michalski et al., 2004). Common salts/evaporites found in the Atacama desert include halite, gypsum, anhydrite, borates, sodium sulphates and perchlorate salts (Stoertz and Ericksen, 1974; Ericksen, 1981; Böhlke et al., 1997; Flahaut et

al., 2017). The Atacama desert has an average temperature of 11-16°C with strong fluctuations during day or night time, and with elevation (Stoertz and Ericksen, 1974; McKay et al., 2003; Clarke, 2006; Gómez-Silva et al., 2008). It is thought to be the oldest desert on Earth, with extreme arid conditions persisting for at least 10-15 million years (Ericksen, 1983; Berger and Cooke, 1997; Clarke, 2006). The region is also exposed to the highest doses of UV radiation found on Earth (Cordero et al., 2018) and oxidizing conditions are commonly found in the desert soils (Navarro-González et al., 2003). Together, these traits make the Atacama desert one of the harshest environments on Earth, challenging life on many fronts and therefore useful for determining the effects of Mars-like conditions on biology and the preservation and detection of life's organic building blocks.

The geological setting of the region is of considerable interest because of its value as an analogue to certain Martian surface environments on top of extreme conditions. Sulfate and chloride-rich deposits found on Mars have similar features as those that are found in the salars of the Atacama desert and may have formed via comparable processes (Sutter et al., 2007; Flahaut et al., 2017). Whereas the western section of the Atacama desert lies over a sedimentary bedrock, the Andean section to the East, which encompasses our sampling area, is made of a variety of basaltic to dacitic volcanic rocks which might be somehow similar to rocks of the martian crust (Flahaut et al., 2017).

Still, current conditions on Mars are typified by extreme aridity, low temperatures (average −60 °C), oxidizing surface conditions and high radiation levels due to a thin atmosphere (Pavlov et al., 2002; Quinn et al., 2013; Hassler et al., 2014), and are much more severe than in the Atacama desert. These conditions would generally greatly challenge the potential presence of carbon-based life, especially near the surface where radiation and oxidizing conditions are most extreme. However, evidence exists that supports the hypothesis that Mars had a more favorable climate (at least) during the first few hundred million years after its formation with warmer temperatures and large bodies of liquid water (Pollack et al., 1987; Squyres et al., 2004; Poulet et al., 2005; Bibring et al., 2006; Mustard et al., 2008; Ehlmann et al., 2011). This evidence includes the detection of a multitude of (localized) hydrated minerals, including phylosillicates, sulfates, carbonates and chlorides, which generally only form in aqueous environments (Gendrin et al., 2005; Poulet et al., 2005; Bibring et al., 2006; Osterloo et al., 2008; Murchie et al., 2009). Overall, there appears to be a widespread presence of water-lain sedimentary rocks, thought to have formed in shallow seas, lakes and salars (Carr and Head, 2010; Hynek et al., 2010; Grotzinger et al., 2014; Flahaut et al., 2015). Further evidence includes observations of a large number of apparent ancient river channels and valleys, which also strongly suggest that the flow of large bodies of water was once prevalent here (Carr, 1996; Carr and

Head, 2010). However, it is thought that a climatic shift of planetary scale has changed Mars into the hostile planet such as we know it today. An additional interesting feature of the Atacama desert is that it has also undergone a shift from wetter conditions in the past, to the current extreme aridity. Studies on the lithology of the region have described different depositional environments from the late Triassic up until today which are summarized by *Clarke et al.* (Clarke, 2006). Depositional environments during the Triassic include fluvial landforms overlain by marine sediments and alluvial fans, while the early Jurassic and Cretaceous are typified by marine to evaporitic marine environments and coastal saline lagoons, respectively. During the late Creataceous-Paleocene, westerly derived alluvial fans fed waters into playa lakes. At the time of the Oligocene-Middle Miocene the region was overlain by a continental evaporitic lake which gradually changed into a continental playa lake towards the Plio-Pleistocene. Today, the region is typified by extreme aridity with scattered evaporitic lakes. This shift is largely recorded in the local bedrock and could also provide insight in processes that may have taken place on Mars as well.

The goal of this study is to investigate several of the salars in the Atacama desert region with regard to their biological fingerprints. The driest parts of the Atacama region have long been thought to be devoid of life (Navarro-Gonzalez et al., 2003). However, the past 15 years have shown that even in the driest regions of the desert, microbial communities exist that found ways to survive and even thrive under these extreme conditions, sometimes which a patchy distribution (Warren-Rhodes et al., 2006; Lester E.D., 2007; Parro et al., 2011; Patzelt et al., 2014). Furthermore, organic carbon has been shown to be present only in low abundances, which forces another limitation on the sustainment of microbial life in the region (Azua-Bustos et al., 2017). We will survey here the distribution of biological fingerprints at distinct locations which show different rock composition, elevation, temperatures and proximity to water ponds.

A recent study by *Flahaut et al.* (Flahaut et al., 2017) has focused in great detail on the mineralogy and geological settings of several such salars in the Atacama desert by using space-born, field (VNIR spectroscopy) and laboratory based analysis. During fieldwork, samples for biological analysis were collected which were analyzed in the study presented here. The four salars that were investigated during this study were all located within the Antafogasta region of Northern Chile (Figure 1). These salars include the Salar de Atacama, which is the largest salar and located on sedimentary basement and was formed at intermediate elevations (2’500 m) under hot and dry conditions. The other 3 salars investigated in this study are located in the volcanic Andean highlands, at higher elevations (4’000 m)

and with slightly wetter and cooler conditions. For more details on the geological setting and mineralogical content of these salars we refer to the paper of *Flahaut et al.* (Flahaut et al., 2017).

Using Illumina 16S amplicon sequencing techniques, we found versatile microbial communities present within the investigated salars, being dominated by (extremely) halophilic organisms. Amino acids, organic carbon and nitrogen were also determined and together, our results indicate that the investigated salars can be considered bastions of life in one of the harshest environments on Earth. Therefore, areas on Mars showing similar features as the salars investigated in this study could be interesting targets for life detection missions heading for the red planet.

2 Materials and Methods

2.1 Sampling sites Description

The sample material investigated in this study was collected from the Andean highlands (altitude of about 4'500 m) and intermediate elevation sites (altitude up to 3'000 m) of the Atacama Desert, Chile. Four different salars (Salar de Laco, Laguna Tuyajto, Salar de Aguas Calientes 3, and Salar de Atacama) were sampled at their center and margins during a field expedition led by the VU University of Amsterdam in March 2015 (Figure 1). Samples of salt crusts and/or wet sediments were collected both at the surface and shallow depths (20-30 cm), when possible (Figure 2). In Tables 1 and 2 a short description and GPS coordinates of the field sites and additional information of the sample material, including e.g. number of samples collected at each field site can be found. Figure 1 shows the specific locations at which sample material was collected. A detailed discussion of the field sites and the mineralogy of the investigated material can be found in (Flahaut et al., 2017).

2.2 Sample Preparation and Methods

2.2.1 Amino Acid Extraction

The amino acid extraction procedure is explained in the following briefly. Detailed information can be found in (Botta et al., 2002). For sterilization reasons all glassware, including the columns with glass wool were double wrapped in aluminium foil and placed into a furnace at 500°C for a minimum of 3 h. Serpentine powder was sterilized as well at this temperature and used as internal control to track potential contaminations during the extraction procedure. Rock and sediment samples were dried in a centrifuge-dryer system (CentriVap Centrifugal concentrator/ColdTrap system, LabConco) before grinding them to fine powder in ceramic mortars with pestles in a

laminar flow. 200 mg of powder material of each sample was transferred into glass tubes and 3 ml of ULC/MS pure grade water was added. The glass tubes were subsequently flame sealed and placed into a heating box at the set temperature of 100 °C for the next 24 h. After heating the tubes were opened and centrifuged at 2'500 rpm for 10 min to separate the supernatant from the solid part (total volume of supernatant 3 ml). Half of the supernatant (1.5 ml) was placed in 5 ml glass test tubes (labelled as H, for hydrolysis). The other half supernatant was supplemented with 1 ml of ULC/MS pure grade water, vortexed and centrifuged again at 2'500 rpm for 10 min, after that all the 2.5 ml of the supernatant was transferred into 5 ml glass tubes and labelled as NON-H (not hydrolised). All the samples are subsequently dried within the CentriVap/Coldtrap system. After drying, the glass tubes with the samples labelled as H (3 ml) were placed inside bigger glass tubes (20 ml) with 6M HCL at the bottom, flame sealed again and placed into an oven at 150 °C for 3 h to undergo acid hydrolysis. Afterwards, these tubes were dried for 1 h using the CentriVap/Coldtrap system.

3 – 4 ml of a resin solution (100 ml ULC/MS pure grade water + 24 mg of AG®50W-X8 Resin, Analytical grade 100-200 mesh purchased from Bio-Rad) was subsequently added to the columns with glass wool. A sequential washing with basic-neutral-acid-basic solutions was made to activate the resin active sites. The basic solution (up to pH of 14) was a 2M Sodium Hydroxide solution (8 g of NaOH in 100 ml of ULC/MS pure grade water), and the acid solution was a 1.5M HCL acid solution (12.5 mL HCL in 87.5 ml of ULC/MS grade water). ULC/MS grade water was used for neutralisation after basic and acid washing, until a pH of about 7 was reached. After the sequential washing procedure, 3 ml of ULC/MS pure grade water was added to each dried sample, and subsequently added to the column. The elution was collected and stored at about 4°C for further analysis. 5 ml of a 2M ammonia solution (ULC/MS grade water to 15.304 mL of 25% ammonia for a final volume of 100 ml) was added subsequently to the columns to remove the amino acids bound to the resin. The eluent was collected using glass tubes, and the ammonia was removed overnight using the CentriVap/Coldtrap system. The dried samples were either stored in a fridge or freezer or directly reconstituted in 100 µl ULC/MS grade water for subsequent analysis (see next sections).

2.2.2 Liquid Chromatography Mass Spectrometry

The system used for amino acid analysis is an Agilent 1260 LC-MS system equipped with an ultraviolet (UV) and fluorescence (FL) detector system, an autosampler module where the amino acid derivatization is performed, and a

500-MS ion trap mass spectrometer with electrospray ionisation. The column used for analysis was a 150 x 3mm 2.6 µm phenyl-hexyl stationary phase from Phenomenex which was thermostatted at 25 °C. The LC was operated in a binary gradient of mobile phases A (10mM ammonium acetate in ULC/MS water) and B (ULC/MS grade Methanol). The MS was operated in positive mode with optimised conditions for each individual amino acid. Amino acids were derivatized using a method based on (Nimura and Kinoshita, 1986) which was then fully automated in order to increase the robustness of the method. This automation was achieved by programming the autosampler module (Agilent G1329B) of the HPLC to mix the various reagents. The approach used was as follows: the amino acid sample was diluted in a 1:1 ratio to yield a solution of amino acids in 0.1 M HCl and a set amount of internal standard (usually 100 mM Norvaline). This was then mixed in a 1:3 ratio with 0.1 M sodium borate, this mixture was then mixed in 2:1 ratio with methanolic OPA/NAC (8 mg OPA and 10 mg NAC in 1 mL methanol), this mixture is incubated for 2 min after which 2.5 µl is injected into the system. In a typical measurement run several samples were analysed sequentially, including wash procedures and the analysis of amino acid standard solutions (Agilent, part number: 5061-3332). Amino acids were identified by comparison to retention times of known standards as well as their associated m/z ratio for the derivatized molecules. Standards were run in conjunction with sample analysis in order to track reagent degradation and system performance.

2.2.3 Gas Chromatography Mass Spectrometry

Amino Acid Derivatization

A water extract (25 µl) was transferred into a reaction vial, the total volume was adjusted to 50 µl with 0.2M HCl solution and a 2,2,3,3,4,4,4-heptafluoro-1-butanol/pyridine mixture (3:1, *v/v*; total volume 25 mL) followed by 5 µl of ethyl chloroformate was added. The vials were capped tightly and shaken vigorously for 10 s to form ECHFBE derivatives. Finally, the ECHFBE derivatives of amino acids were extracted into the organic phase from the reactive mixture by adding 50 µl of methyl laurate in chloroform (10^{-5}M), the latest served as internal standard. The organic phases were withdrawn and transferred into 1 ml GC vials equipped with 100 µl inserts for enantioselective GC×GC analyses.

Multidimensional Gas Chromatography Mass Spectrometry

The enantioselective multidimensional analysis was carried out by GC×GC Pegasus IV D coupled to a time-of-flight mass spectrometer (LECO, Michigan, USA). The mass spectrometric system operated at a storage rate of 150 Hz,

with a 50 − 400 amu mass range, detector voltage of 1.8 kV, and solvent delay of 15 min. The ion source and injector temperature were set at 230 °C. In all runs the column set consisted of a Chirasil-L-Val column (24.885 m × 0.25 mm, 0.12 µm film thickness) in the first dimension and DB Wax as secondary column (1.19 m × 0.1 mm, 0.1µm film thickness) coupled to a modulator (0.21 m × 0.1 mm, 0.1 µm film thickness). The maximum operating temperatures of the columns were 200 °C and 250 °C, respectively. Helium was used as carrier gas at a constant flow of 1 ml/min. All samples were injected in the splitless mode applying the identical temperature program. The temperature of the primary column was held at 40 °C for 1 min then it was increased to 80 °C at a rate of 10 °C min^{-1} and held at this temperature for 5 min, finally it was heated up to 180 °C with 2 °C min^{-1} rate and held at this point for 7 min. The secondary oven used a temperature off set of 30 °C. It was held at 70 °C for 1 min, warm up to 110 °C with 10 °C min^{-1} rate and held at this temperature for 5 min, then heated up to 160 °C at a rate of 2 °C min^{-1}, finally it was increased to 220 °C at rate of 4 °C min^{-1} and held for 22 min. A modulation period of 5 s was applied. The derivatised soil and serpentine extracts as well as corresponding replicates were injected 3 times to ensure accuracy of calculated peak area with reliable statistical error bars. Data were processed using the LECO Corp ChromaTOF™ software. Volume peak integrations were performed, taking into account possible modulation-induced errors (Meinert and Meierhenrich, 2012).

2.2.4 Illumina 16S amplicon sequencing

DNA extraction

Rock and sediment samples were dried in an oven at 80 °C overnight and subsequently powdered with sterile mortar and pestle (DNA-away solution, heat sterilisation and UV sterilisation). The DNeasy PowerSoil extraction kit (Qiagen, Hilden, Germany) was used for the DNA extraction of the samples. Because of an expected low biomass, an adapted protocol was used to minimize sorption of DNA by the mineral matrix (Direito et al., 2012). Furthermore, the DNA extract was eluted in 70 µl instead of the regular 100 µl to further increase the concentration of the extract. All handlings of the samples and negative controls during the extraction process were conducted in a UV3 HEPA PCR workstation (UVP, Upland, CA, USA), equipped with a HEPA filter and a UV illuminator to prevent extraneous DNA contamination. Concentrations of the extracts were determined using a Quant-iT high-sensitivity DNA assay kit and a Qubit® fluorometer (Invitrogen, Carlsbad, USA). DNA extracts were stored at -20 °C until further processing.

Illumina 16S amplicon sequencing

All DNA extracts were diluted to a concentration of 0.1 ng/µl prior to PCR amplification. If the concentration of the DNA extract was lower than 0.1 ng/µl it was processed undiluted. PCR reactions were performed in triplicate using Phusion Green Hot Start II High-Fidelity DNA Polymerase (Thermo Fisher Scientific, Sweden). We targeted the V3-V4 region of the 16S rRNA gene, using the V3 forward primer S-D-Bact-0341-b-S-17, 5'-CCTACGGGNGGCWGCAG-3 (Herlemann et al., 2011) and the V4 reverse primer S-D-Bact-0785- a-A-21, 5'-GACTACHVGGGTATCTAATCC-3' (Muyzer et al., 1993), giving rise to ~430 bp long dsDNA fragments. The primers were dual barcoded and were compatible with Illumina sequencing platforms as described previously (Caporaso et al., 2011). Performance of the PCR reaction was checked by running incorporated positive and negative controls from each triplicate plate on 1.5% (w/v) agarose gels. Triplicate PCR products were combined and each combined triplicate sample was purified using SPRI beads (Agencourt® AMPure® XP, Beckman Coulter, CA, USA). The DNA concentration in the purified samples was determined as described above. Samples were diluted to identical concentrations of 2 ng/µl prior to pooling the diluted PCR products together in equal volumes (10 µl) in one composite sample (including positive and negative controls).

The composite samples were paired-end sequenced at the Vrije Universiteit Amsterdam Medical Center (Amsterdam, The Netherlands) on a MiSeq Desktop Sequencer with a 600-cycle MiSeq Reagent Kit v3 (Illumina) according to manufacturer's instructions. High-throughput sequencing raw data were demultiplexed using bcl2fastq software version 1.8.4 (Illumina) and primers were trimmed using Cutadapt (Martin, 2011). Demultiplexed samples were further processed using a modified version of the Brazilian Microbiome Project 16S profiling analysis pipeline (Pylro et al., 2014). Paired-end reads were joined using PANDAseq (Masella et al., 2012) selecting for a minimum overlap of 30 nucleotides between the forward and reverse reads. We selected for a minimum sequence length of 285 bp and no mismatches in the primer region were allowed. PANDASeq addresses mismatches in overlapping regions by selecting the nucleotide with the best sequencer-assigned quality score. Because PANDAseq incorporates a base quality filter during read assembling, the threshold for consecutive high quality bases per read was set to zero. Metadata and demultiplexed samples were merged using add_qiime_labels.py (Caporaso et al., 2010) and sequence headers were changed using bmp-Qiime2Uparse.pl (Pylro et al., 2014). UPARSE was used to dereplicate, filter chimeras, discard Operational Taxonomic Units (OTUs) detected less than 2 times and OTU clustering at 97% similarity (Edgar, 2010; Edgar, 2013). The OTU taxonomy was assigned using the UCLUST algorithm (Edgar,

2010) on QIIME (Caporaso et al., 2010) using SILVA compatible taxonomy mapping files (Silva database release 128) (Quast et al., 2013; Yilmaz et al., 2014) and aligned using align_seqs. py in QIIME (Caporaso et al., 2009). Taxonomy was manually curated and refined up to genus level based on 97% similarity of reference sequences. The reference tree was calculated using the make_phylogeny script in QIIME (Price et al., 2009). We generated a BIOM file using make_otu_table.py on QIIME (Caporaso et al., 2010). Prior to further analysis we produced an OTU table and a taxonomy table using BIOM scripts (McDonald et al., 2012). The OTUs detected in negative controls and procedural blanks were manually removed from the dataset. After quality filtering, taxonomy assignment (97% identity), subtraction of contaminating sequences and removal of singletons, the dataset comprised 731'249 reads which were attributed to a total of 3'900 OTUs. Next, a filtering step was performed as described by *Bokulich et al.* (Bokulich et al., 2013), where OTUs representing fewer than 0.005% of the total reads were removed from the dataset. This trimmed dataset resulted in 700'874 reads and a total of 1'404 OTUs. This dataset was rarefied choosing 1'000 reads as the minimum count in order to include 16 of the 17 samples resulting in a dataset comprising 16'000 reads and 1'135 OTUs.

2.2.5 Carbon and nitrogen measurements

Samples were powdered using heat sterilized mortar and pestle and subsequently dried at 60 °C for 24 h. Analysis was carried out using a FlashEA 1112 (Thermo Scientific), with EAGER300 operating software and a thermal conductivity detector (TCD). For carbon and nitrogen measurements, approximately 30 mg of each sample was weighed in silver holder cups. An empty holder cup was used as a blank. 2-3 mg of Ethylenediaminetetraacetic acid (EDTA) was used as standard (7.522 % N, 32.237 % C, 4.836 % H, 34.386 %). For the removal of inorganic carbon, cups containing the samples were placed upright in a desiccator containing 12M HCl. Samples were exposed overnight to the vapors. Complete removal of inorganic carbon was tested by adding 10 µl of 10% HCl to a cup in order to determine if vapors were produced (which would indicate presence of more inorganic carbon). This procedure was repeated until no further fumes were produced. Samples were dried at 80 °C prior to CN measurements. The silver cups were sealed off and placed within a tin cup, which was sealed prior to measurement. Abundances are measured as percentage per weight and measurements were carried out in duplicates.

3 Results and Discussion

In the following, we show and discuss the biological fingerprints we obtained from the different rock and sediment samples from the four different salars. By combining the results obtained from different measurement techniques, and by taking into account the environmental differences between the sampling sites we aim to create an overview of the microbial life and its subsequent building blocks which thrives in these types of salars and how this is relevant for search of life on Mars.

3.1 LC-MS and GCxGC-MS Analysis of Amino Acids

Amino acids were measured in order to obtain a general overview about the biological load in the soil samples. The soil samples were divided in two different sets (ATA01-ATA09 and ATA10-ATA17) and these sets were analyzed by different measurement techniques (LC-MS and GCxGC-MS, respectively). Due to the use of different techniques and standards, some amino acids were not measured for both sample sets and the D-amino acids were only determined by GCxGC-MS. Therefore, we will mainly focus on the amino acids that were determined by both measurement techniques. Amino acids are considered high priority targets in the search for extraterrestrial life since they make up the building blocks of the entire protein machinery of life on Earth but are also commonly found in meteorites (Ehrenfreund and Charnley, 2000; Martins, 2008; Aerts et al., 2016), indicating these building blocks of life are common organic compounds in our Solar System.

Amino acids were detected in readily detectable quantities in most samples, in highly variable amounts (Table 3). A few samples have considerably lower abundances of amino acids. These include one sample collected from the drier central part of Laguna Tuyajto (ATA08) and two samples from Salar de Atacama (ATA15 and ATA17). These amino acid values are more similar to those of samples obtained from the driest region in the Atacama desert (Yungay) which were previously analyzed (Skelley et al., 2005; Amashukeli et al., 2007). However, overall we detect relative high concentrations of amino acids if compared to other regions in the Atacama desert and this is likely linked to the presence of surface water and / or more pronounced presence of life that is found in the Andean altiplano.

Samples collected from Salar de Laco (ATA01-ATA04) all show similar abundances of total amino acids, whether they were collected at the margin or at the center of the salar. Both locations show similar composition with a high proportion of dentritic material. Distributions of the individual amino acids differ somewhat (i.e. Glycine is much

more dominant in ATA03 and ATA04, while Methionine is abundant in ATA01 and ATA02 but virtually absent in the other two samples) but overall most amino acids are present in a similar order of magnitude.

Samples from Laguna Tuyajto showed some more pronounced differences between the sampling locations in terms of total amino acid content. ATA05, which was taken at the external margin of the salar and close to the water body revealed the highest total amino acid abundances. During DNA extraction, this sample was also observed to have a high DNA load (Figure 4). ATA07 and ATA08 were sampled at the center of the salar with no nearby presence of water and were composed of pure salts. These samples showed lower abundances of amino acids, while ATA09 (sampled between the external margin and the center) had amino acid abundances in the range somewhere in the middle between the other two sample locations in this salar.

Samples ATA10 and ATA11 from Salar Aguas Calientes 3 had by far the highest total load of amino acids of all the samples analyzed (5-10x higher than other samples with significant amino acid concentrations). These samples were collected on a pebble beach part close to a water body, which may explain the increased abundances. Organic materials dissolved in the water are likely to seep into the surrounding soils, or have left behind organic molecules during a time of higher standing water. Samples ATA12 and ATA13, taken closer to the center part of the same salar, had much lower abundances of amino acids.

Very low concentrations of amino acids were found in sample ATA14 from Salar de Atacama. This sample was also excluded from the DNA sequence analysis due to a lack of sufficient amplifiable genomic material in the DNA extract. ATA17, another sample from the same salar, thick and dry halite core, did not reveal the presence of amino acids above the detection limit. This sample also contained no detectable DNA in the original extract, however after PCR amplification enough material was found for sequence analysis. ATA15 and ATA16 have the highest amino acid loads of samples from Salar de Atacama. The sampling had significant vegetation, which may explain higher amino acid abundances. In both of these samples, genomic material was detected in the original extract.

Proteinogenic amino acids are by far the most abundant types we found, and enantiomeric ratios (L-amino acids have higher abundances than their D-amino acids counterparts) clearly indicate biotic origin of these molecules (Uwe J. Meierhenrich, 2008). The amino acids that are detected in high abundances in the investigated soil samples all fall in the category of most dominant amino acids in the protein machinery of the three kingdoms of Archaea, Bacteria and Eukarya (Kozlowski, 2016). Furthermore, a recent report by the NASA, concerning the lander mission

on Jupiters moon Europa (Hand et al., 2017), specifies seven amino acids (Ala, Gly, Leu, Ser, Val, Asp, Glu) as important 'biomarkers' due to their relative high abundance in biological systems on Earth as well as in extraterrestrial samples such as carbonaceous meteorites, suggesting these molecules are widely spread throughout our Solar System. All these seven amino acids are detected in relative high quantities in these samples. Next to these seven amino acids we also detected Phenylalanine and Iso-Leucine in high quantities with strong enantiomeric excess of the L-isomer. Although less common in carbonaceous chondrites, these two amino acids could also be considered interesting biomarkers (as would be all proteinogenic amino acids) since their presence would point towards more complex (i.e. biotic) synthesis processes. However, presence of amino acids should clearly not be considered a signature of life on its own. Distribution patterns and enantiomeric excesses should also be considered.

When looking at the relative contribution of the nine most dominant L-amino acids per sample, some variation between samples is observed (Figure 3). Most notably, samples ATA08 and ATA14 deviate from the other samples. This is likely connected to the relative low total amino acid load detected in these samples. Furthermore, it is interesting to note that specifically samples from Salar de Laco have relatively high contributions of Phenylalanine, while this is mainly a minor contributor in samples from the other salars, where generally Glycine is the most dominant amino acid. Although there are marked differences in amino acid distribution between the different samples, 'fingerprints' as observed here can serve as strong biomarkers. Even though there are differences, the general constitution of these samples does not appear random and a certain trend of dominant and less dominant amino acids is observed within most samples. Distributions of amino acids produced by abiotic processes differ significantly from distributions that arrive from biological systems which is mainly caused by the importance of function in the latter as opposed to 'random' thermodynamic processes that influence the formation of the former (Higgs and Pudritz, 2009). It may therefore be more fitting to focus on relative contribution within a sample, rather than focusing on the quantification of individual amino acids.

3.2 Microbial community analysis

DNA was extracted from the soil samples to determine the composition of microbial communities present within the soil samples using Illumina 16S amplicon sequencing. The main goal of DNA analysis was to determine which type of microorganisms are present in environments as these. DNA quantities in the soil were overall low but in the majority of the samples detectable DNA was present (Figure 4), while in some samples DNA load was rather high (notably ATA05, of which the analyzed sediment had a greenish color). This suggests that in most of the investigated soils, microbes (or plant life) are present in easily detectable numbers without the need for amplification techniques. Furthermore, surface and subsurface samples were taken at most sampling sites (Table 2) and although it would generally be expected that subsurface samples would result in the recovery of lower biomass due to the absence of photosynthetic processes, there is no such trend observed here. This is likely due to the low depth from which samples were acquired (10-25 cm), and may not be very representative for future Mars missions (i.e. ExoMars) that aim to drill to a depth of 2 m for sample collection (Vago et al., 2017). However, due to strong UV radiation and high evaporation rates at the surface in this specific region, microbial life may endure better at depth. Evidence for biological material on several sites was also found previously in the form of chlorophylls, carotenoids and pigments using VNIR and Raman spectroscopy (Flahaut et al., 2017).

Sequencing results revealed the presence of diverse prokaryotic communities, and a total of 1’404 Operational taxonomic units (OTUs) were found after removal of contaminating sequences and filtering steps. The dataset was rarefied (i.e. the same number of sequence reads were picked from each sample at random) in order to create equal library sizes for the samples in downstream analysis so that samples can be compared in terms of relative abundances of the different putative species present. Generally, rarefaction results in dismissing only the least abundant OTUs (operational taxonomic units) from the dataset. The number of OTUs per sample shows that there is quite some diversity in the number of species, whether they came from the same area or from different sampling sites (Figure 5). Higher initial concentration of DNA in the extracts did not per se result in a higher number of species in the sample communities, although samples with the highest initial DNA load tend to have relative high alpha diversity (i.e. samples ATA05, ATA06 and ATA16). Sample ATA14 was excluded from the rarefied dataset because there were not enough sequence reads to include it in the analysis (low biomass). This sample was collected at the Salar de Atacama, which is the driest and hottest region of the different sampling locations. Samples from Laguna Tuyajto show the highest numbers of species within the soil, followed by samples from Salar de Laco, while samples from Salar de Atacama and from Salar de Agua Calientes 3 show on average significantly lower numbers of

species. Laguna Tuyajto was shown by *Flahaut et al.* to have the highest mineral diversity of the four salars along its wet margins which may be reflected in the alpha diversity we observe here. This salar, as well as Salar de Laco are both derived from SO_4-rich brines and show generally the highest species diversity. Salar de Atacama and Salar de Aguas Calientes 3 were shown to have relative thick halite crusts that were both derived from mixed Ca/SO_4-rich brines and were shown to have lower average alpha diversity.

The composition of the microbial community in each sample is shown at the phylum level in Figure 6. Members of the extremely halophilic archaeal family *Halobacteriaceae* (Phylum *Euryarchaeota)* dominate the dataset at family level making up for 39% of the total number of sequence reads. Some of the most dominant genera from this family are: *Haloparvum, Natronomonas, Halorubrum, Halomicrobium and Haloarcula*. However, a total of 36 different genera of this family were present within the dataset. The *Halobacteriaceae* family members are present in higher numbers in the samples in which higher temperatures were measured and are also extremely dominant in the three analyzed samples taken from Salar de Atacama and one surface sample of Salar de Aguas Calientes 3 (ATA12), which was described as having a pinkish mud surface (the pink color likely hails from the distinct carotenoids that members of the *Halobacteriaceae* family produce). A recent study by *Cubillos et all.* (F. Cubillos et al., 2018) determined the prokaryotic communities in highly saline natural brines in the Salar de Atacama (close to our sampling sites) and found that these brines were strongly dominated by *Halobacteriaceae* with strong phylogenetic diversity, which matches well with our own observations. Interaction between these brines and the surface area may locally induce very high salinity, resulting in the more dominant presence of the extremely halophilic Halobacteriaceae species we detected in these sediments. Together with the *Euryarchaeota*, the phyla *Proteobacteria* (18%), *Firmicutes* (13%) and *Bacteriodetes* (11%) contribute to about 80% of all the OTUs in the dataset. Members of the *Halobacteriaceae* family are often seen to dominate in highly saline environments (Mora-Ruiz et al., 2018) and have previously been proposed as viable candidates when looking for (past) life on Mars due to their metabolic diversity and coping strategies with excessive salts (Oren, 2014).

Another extremely halophillic microorganism present in relatively high abundance was a bacteria of the genus *Salinibacter* (*Bacteriodetes*) making up almost 5% of the total number of reads. This species was found predominantly in the surface samples and not so much in samples taken at increased depth. The most dominant genus found in the dataset was a *Bacillus* (*Firmicutes*) species (9%), which is likely a sporeformer which allows it to survive harsh conditions for extended periods of time. This species was present in most of the samples but was

exceptionally prominent in two samples of the central part of the Salar Laco (ATA01 and ATA02). At the center, the salt crust is dominated by gypsum and generally thicker and drier than at its margins, where detritic components such as anorthite and water are more prominent (Flahaut et al., 2017). Generally, the extraction of DNA from sporulated microorganisms is inefficient due to their resistance to extraction techniques. Therefore, finding such high numbers of a bacillus species in the dataset could indicate that they are either in an active non-sporulated state, or that they are even more abundant in the microbial communities and that only a fraction of their DNA was extracted. Another prominent species found in the dataset is an uncultured member of the *Ectothiorhodospiraceae* (*Proteobacteria*) family, part of the purple sulfur bacteria. These organisms are capable of photosynthesis and mostly oxidize sulfite or thiosulfate under anoxic or microoxic conditions to elemental sulfur. These types of organisms are usually found in permanently stratified (often saline) lake environments such as the salars investigated here (Hunter et al., 2008). Furthermore, an uncultured species of the order of *Acidimicrobiales* (*Actinobacteria*) makes up 3% of the total number of reads (mainly due to the high presence in one sample) and several species of extremophiles belonging to the Phylum *Deinococcus-thermus* make up another 2.5% of the total number of reads.

Interesting to note is that one sample taken from the subsurface (ATA04) has no contribution at all from members of the *Halobacteriaceae* family and appears to have a very different microbial composition, which is dominated by an uncultured member of the *Acidomicrobiales* order. Other dominant species in this sample are from the phyla *Chloroflexi* , more*Actinobacteria* and a *Marinobacter* species. Most dominant species in this specific sample are only present (if present at all) in low abundances in the other investigated soil samples, making this particular sample rather unique. This is also observed in the principle component analysis plot in Figure 7 where ATA04 is strongly separated from all other samples based on its species composition. The sediment sample was taken at 20 cm depth from a compact pure calcite crust which was covered by two distinct clay layers. *Flahaut et al.* proposed that Salar de Laco used to be a lake with possibly alkaline waters that progressively dried out and became more acidic. This was found to be consistent with previously reported aerial imagery that identified ancient shorelines in this area. Patchy distribution of sedimentary features of this ancient environment in the subsurface could explain why the microbial composition of this particular sample, but not all samples from Salar de Laco, was so affected.

Overall, there appears to be potential for a wide mix of metabolisms based on the 16S data, which include a multitude of organisms capable of sulfur and nitrogen metabolism, photosynthesis, heterotrophic as well as autotrophic and chemolithotrophic metabolism and aerobic, anaerobic or microaerobic respiration. This observation

shows that even though these salars are considered extreme environments on its own, they are still capable of supplying the necessities for complex and versatile microbial communities to exist. The relative composition of the microbial communities in most samples follows a power-law distribution that is typically found in most soil samples, showing a relative small number of highly dominant OTUs and a large number of low-abundant OTUs (Mandakovic et al., 2018). The most dominant members of the communities observed in the salars are putative species that possess characteristics that would be expected in these environments such as extremely halophilic and acidophilic traits, spore-forming capabilities and/or resistance against radiation and high or low temperatures.

A study focusing on the lake waters and the surrounding sediments of a salt lake in close proximity to the salars investigated in this study revealed dominant bacteria from the taxa of *Bacteriodetes*, *Proteobacteria* and *Firmicutes* (Mandakovic et al., 2018). No primers were used to amplify archaeal DNA. It is interesting to note that in terms of bacterial abundances, we also see these taxa as the dominant contributors to the bacterial communities. Furthermore, many of the putative species detected in our study were also found in the study by *Mandakovic et al.* and overall the relative abundances are similar. This seems to further suggest that microbial communities in the sediments of different salars in the region show strong similarities in terms of composition, which was also observed between the salars investigated in this study.

In order to determine whether the salars harbor distinctively different microbial communities, principle coordinates analysis was performed (Figure 7). Some cluster formation is observed for the different salars, however there is also considerable overlap and sometimes samples (i.e. microbial communities from different salars are more similar than samples from the same salar). The three Salar de Atacama samples (Peine Road) form a more pronounced separate cluster as compared to the bulk of the other samples, suggesting that local conditions in this salar affects the microbial composition of the communities. However, samples from Salar de Laco and Salar de Aguas Calientes 3 also group within this cluster, and all have very high contributions of Halobacteriaceae species. Using Adonis tests it was found that sampling location (i.e. the salar from which the sample was taken) ($p = 0.028$; $R2 = 0.4$), sampling depth ($p = 0.015$; $R2 = 0.21$) and the soil temperature ($p = 0.002$; $R2 = 0.47$) had significant effects on the variation and the abundances observed within the samples microbial communities. Effect sizes (R^2) quantify the amount of variation explained by the chosen parameter (i.e. sample location, sampling depth, soil temperature) in its respective

ordination. So in their respective ordinations we find that 40% of the observed variation can be explained by sampling location, 21% by sampling depth and 47% by soil temperature. However, the combined effects of these three measured variables (and likely more hidden variables) result in an image that suggests that regional, as well as local and small scale factors influence distribution of microbial communities. No significant effect was found when looking at whether the sample was taken at the (often wetter) margin or the centre of the salar. In the accompanying paper focusing on the mineralogy of these salars (Flahaut et al., 2017), it was found that different mineralogy dominates at the centre of the salars (salts) if compared to the margins (more detrital material, carbonates and clays). This does not appear to significantly affect the composition of the microbial communities on its own and is likely linked to the mixed nature of many of the sediments. In general the species distributions of the different samples did not follow distributions in the quantitative presence of halite or gypsum that were determined for the sediments by *Flahaut et al.* (2017).

3.3 Organic Carbon and Nitrogen content

Overall, low concentrations of organic carbon and nitrogen were measured (Figure 8), with carbon measurements typically (far) below 0.5 weight percentage and nitrogen load an order of magnitude lower (resulting in C/N ratios varying between 8 and 14). The low organic carbon content in most samples is in line with the expectations due to the region being notorious for having low carbon (Lester E.D., 2007; Azua-Bustos et al., 2017). Especially samples ATA01, ATA02, ATA12, ATA14 and ATA17 have extremely low carbon (0.05-0.11 % weight) and are comparable to soils from the most arid region in the Atacama desert (Yungay) in terms of their organic carbon load (Lester E.D., 2007). In four of these five extremely low carbon samples, also no measurable DNA (below detection limit) was detected in the original extract prior to PCR amplification. There was only one other sample that contained no detectable DNA (ATA09, which also had fairly low carbon: 0.19 %). Furthermore, ATA05 and ATA06, having significantly higher carbon than the other samples, were sampled at the margin of their respective salar and were relatively close to a water body. The presence of water may partially explain their higher carbon and nitrogen content which correlates with the presence of more pronounced life. This is also observed in the DNA extracts where especially ATA05 and to a lesser degree ATA06 show relatively high concentrations of DNA. Although not quantitatively, there is a trend observed where organic carbon values in the soil appears to reflect initial extractable DNA (whether this is prokaryotic or eukaryotic DNA). The largest fraction of the OTUs that are detected in the

sequence results belongs to heterotrophic organisms, which rely on the presence of organic carbon for biomass production. Therefore, stronger presence of carbon in the soils would allow microbes to grow to increased densities, resulting in a higher retrievable fraction of DNA. However, Adonis tests showed that concentrations of organic carbon and nitrogen in the samples did not have a significant effect on the type of species present in the communities.

Organic carbon and nitrogen show a different distribution compared to the abundances of the amino acids in the soil samples. High amino acid load does not necessarily result in higher carbon or nitrogen values. This is likely due to the fact that amino acids only form a smaller fraction of the organic compounds that are present in most soils and therefore their varying abundances are obscured. The weight percentage of total amino acids (using an average molecular weight of 120 Da) in our samples was found to be on average about 20 times lower than organic carbon values. Considering the contribution of nitrogen, hydrogen and oxygen to the molecular weight of amino acids as well, the contribution of amino acids to total organic carbon will be even lower. This phenomenon could explain that there is no correlation seen between amino acid abundances and organic carbon and nitrogen values.

4 Conclusions

This study contributes to the ongoing expansion of our knowledge regarding the biological load in extreme environments serving as Mars analogues. The salars investigated during this study were previously described as having a strong Mars analogue potential due to the unique arid and volcanic environment of the region as well as the similarities in geological processes (Flahaut et al., 2017). We found here that microbial life was strongly represented in all salars and that the prokaryotic communities match with previous observations. The communities show large contributions of (extremely) halophilic organisms as well as a relatively large number of species involved in the sulphur cycle, which is expected in an environment rich in salts and sulphates (i.e., halite and gypsum). Overall, a wide variety of microorganisms was found in these salars which appear perfectly capable of surviving under the harsh conditions in one of the most extreme environments on Earth. However, there were clear differences observed between sample sites regarding the extractable biomass, suggesting that patchy distribution is still an issue to be considered. Samples with some of the highest biomass signatures were systematically found within relative close proximity to (small) temporary bodies of water in the wetter margin areas.

The patchy nature of these salars did not allow us to appoint typical microbial communities per salar. Overall, different samples (from the same or from different salars) share a large number of species, although the abundances can differ dramatically. At the same time, there are many species (often less abundant) that are selectively represented in a subset of samples, which induces the variation we observe in the ordination plot. Small scale variations in temperature, sediment composition, humidity and sampling depth (among other parameters) likely play a role in the observed variations. However, differences in mineralogy between the sample sites (Flahaut et al., 2017) did not explain the distributions observed in the microbial profiles. This is likely because most samples were seen to contain a mix of similar minerals and evaporites although the relative contributions per site vary. Brine types associated with the different salars may have influenced the number of different species (alpha diversity) that were detected since the salars fed by SO_4-rich brines had higher alpha diversity. SO_4-rich brines in the region likely result from extensive circulation in volcanic rocks in the subsurface while Ca, or mixed brines are proposed to result from circulation in the sedimentary bedrock (which in this area is mostly composed of sandstones and conglomerates) (Risacher et al., 2003). It may therefore be expected that SO_4-rich brines are richer in nutrients and could support a larger number of species (explaining the higher alpha diversity in these salars). However, Adonis tests did not result in significant p-values for the effect of brine type on the microbial distributions observed in the samples, suggesting that other environmental parameters exert stronger effects.

Amino acids and organic carbon and nitrogen found within the investigated soils match the microbial observations in that they generally correlate with the presence of pronounced life. Most samples showed considerable amounts of amino acids of which the proteinogenic amino acids made up the bulk. The presence/absence, distribution, and enantiomeric excesses of these amino acids can be directly linked to the microbial life that is found within the samples, although a quantitative correlation could not be directly deduced. The same holds true for the organic carbon and nitrogen in the soils which, although low in most samples, reflected to a certain extent the amount of genomic material that was recovered from the soils.

Besides the observation that these salars are shown to support life to a large and diverse degree, there are other features that make these type of environments important targets for future Mars missions. Salts like halite and gypsum, which dominate these salars, have strong preservation potential for biomarkers (Fernández-Remolar et al.,

2013; dos Santos et al., 2016) and have been shown to be present on Mars, often in evaporitic contexts (Osterloo et al., 2010; Flahaut et al., 2014; Flahaut et al., 2015). If conditions on ancient Mars would have allowed for microbial colonization in such environments, detectable remnants of these times may have been preserved in the deeper layers of Martian salt flats. Furthermore, halite and or gypsum depositions would be relatively easy to drill as opposed to harder materials in the Martian regolith and would likely make good targets for future life detection missions to Mars aiming to drill to the subsurface. Searching for organics at greater depths is widely considered a prerequisite, and is also a main objective ESA's ExoMars mission, since the surface of Mars has been exposed to billions of years of cosmic radiation (Pavlov et al., 2002; Hassler et al., 2014).

Due to the freezing temperatures on Mars, liquid water is expected to be present mainly as highly saturated brines. This is another strong argument to focus on environments that are rich in salts when searching for traces of life. High concentrations of salts in water lowers the freezing point substantially to around -50 °C (Brass, 1980; Lide, 2004) and may allow certain analogies to extremely halophilic organisms such as *Halobacteriaceae* to survive in such brines, potentially making these types of environments some of the last potential habitats of current day life on Mars. A member of the *Halobacteriaceae* family has previously been isolated from a hypersaline Antarctic lake (Franzmann et al., 1988) and was shown to grow significantly at sub-zero temperatures (Reid et al., 2006).

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Disclosure statement

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TABLES

Table 1: General description of the four different salars investigated during this study. Information on the different Salars was obtained from Flahaut et al. (Flahaut et al., 2017)

Salar	**Brine type**	**Mean elevation [m]**	**Mean Temp. [°C]**	**Evaporation [mm/yr]**	**Area salar [km^2]**	**Main minerals detections (> 50%)**
Salar de Aguas Calientes 3	mixed	3'950	1	1500	46	gypsum, halite
Laguna Tuyajto	SO_4	4'010	1	1500	2.9	halite, gypsum, anorthite (detritic)
Salar de Laco	SO_4	4'250	1	1500	16.2	gyspum, carbonates, anorthite (detritic)
Salar de Atacama	mixed	2'300	14	1800	3000	Halite

Table 2: Field description of the various sampling locations in the four different salars that were investigated. Samples were collected either at the center of the salar or more towards its margins. The reference sample string was taken from Flahaut et al., 2017.

SampleID	Location ID	Reference	Location	Coordinates	Sample Depth [cm]	Temp. at sample depth [°C]
ATA01	A	J2L1R1Z1	Salar Laco, center	-23.84926°, -67.42459°	Surface	30.0
ATA02	A	J2L1R1Z2	Salar Laco, center	-23.84926°, -67.42459°	17-20	19.3
ATA03	B	J2L1R2Z1	Salar Laco, margin	-23.84876°, -67.42517°	Surface	29.5
ATA04	B	J2L1R2Z2	Salar Laco, margin	-23.84876°, -67.42517°	20	17.1
ATA05	C	J2L2R2S4Z1	Laguna Tuyajto, margin, close to water	-23.95548°, -67.58775°	Surface	25.4
ATA06	C	J2L2R2S6Z1	Laguna Tuyajto, margin, close to water	-23.95548°, -67.58775°	Surface	23.8
ATA07	D	J2L2R3S1Z1	Laguna Tuyajto, center	-23.95332°, -67.59512°	Surface	27.6
ATA08	D	J2L2R3S1Z2	Laguna Tuyajto, center	-23.95332°, -67.59512°	12	16.4
ATA09	E	J2L2R4Z2	Laguna Tuyajto, margin	-23.95965°, -67.59531°	12	19.4
ATA10	F	J5L1R3Z1	Salar Aguas Calientes 3, center	-23.91839°, -67.69846°	Surface	22.5
ATA11	F	J5L1R3Z2	Salar Aguas Calientes 3, center	-23.91839°, -67.69846°	8	16.1
ATA12	G	J5L1R4Z1	Salar Aguas Calientes 3, margin	-23.91834°, -67.69817°	Surface	23.0
ATA13	G	J5L1R4Z2	Salar Aguas Calientes 3, margin	-23.91834°, -67.69817°	24	11.5
ATA14	I	J7L14	Salar de Atacama (Peine Road), margin	-23.65346°, -68.12642°	Surface	48.2
ATA15	J	J7L16Z1	Salar de Atacama (Peine Road), margin	-23.66163°, -68.09688°	Surface	44.9
ATA16	J	J7L16Z2	Salar de Atacama (Peine Road), margin	-23.66163°, -68.09688°	15	29.4
ATA17	H	J7L9Z1	Salar de Atacama (Peine Road), center	-23.68421°, -68.30838°	Surface	43.0

Table 3. Amino acids identified and quantified in Atacama samples using LC-MS and GCxGC-MS. Abundances are given in [nanomol/gram soil]; LC-MS: for displayed values a measurement uncertainty of 10% is applied; GCxGC-MS: displayed values with a confidence interval of 3σ. Only the most abundant amino acids are shown in the table

LC-MS measurements

Compound	ATA01	ATA02	ATA03	ATA04	ATA05	ATA06	ATA07	ATA08	ATA09
L-Asp	BB	4.6636	39.6980	45.4882	22.8467	n.a.	15.9786	BB	20.4145
L-Glu	40.8835	16.6413	139.3258	133.2587	71.6233	n.a.	38.5571	LOQ	35.3504
L-Ser	14.8565	10.5807	34.7016	32.0143	38.8338	n.a.	10.8616	1.6199	14.0343
L-Thr	8.2926	7.2422	35.2993	39.4337	22.2787	n.a.	20.1369	1.5384	24.4815
Gly	28.4561	29.2985	112.5927	130.3262	184.0706	n.a.	32.5197	19.7174	76.5153
β-Ala	2.7706	1.7261	10.0720	14.5592	26.9636	n.a.	1.8149	2.2183	7.1848
L-Ala	14.8567	9.4829	52.6682	60.9958	83.4233	n.a.	27.5205	5.5068	41.2645
L-Lys	26.9343	179.6670	183.3782	6.3372	92.4194	n.a.	BB	BB	61.9660
L-Val	3.5567	25.3343	28.0825	12.5779	21.5742	n.a.	54.8623	1.9426	27.5493
L-Met	70.8579	64.3128	BB	1.0020	BD	n.a.	BD	6.0634	BD
L-Iso-Leu	30.1724	LOQ	22.8995	21.2417	BB	n.a.	27.8112	BB	28.4878
L-Phe	153.4099	294.5115	257.4771	90.9713	7.3644	n.a.	18.1102	1.2591	23.0885
L-Leu	25.4313	40.9702	70.5916	49.6273	27.4641	n.a.	BB	22.0848	12.0110

GCxGC-MS measurements

Compound	ATA10	ATA11	ATA12	ATA13	ATA14	ATA15	ATA16	ATA17
Gly	1370±40	1330±60	240±20	140±11	BB	185±16	195±8	BB
D-Ala	39±4	39±4	LOQ	BB	BB	LOQ	3.12±0.19	BB
L-Ala	510±30	510±30	74±6	46±2	BB	136±6	48±4	BB
β-Ala	28±3	27 ±2	11.6±1.9	BB	BB	BD	9±1	BD
D-Ser	1.39±0.17	1.39±0.17	BD	BD	BD	BD	BD	BD
L-Ser	5.0±0.5	5.0±0.5	LOQ	LOQ	BD	BD	LOQ	BD
D-Asp	211±14	211±14	17.1±1.8	15.1±0.3	LOQ	1.3±0.13	18.4±0.7	BD
L-Asp	610±60	900±60	73±7	66.3±0.9	4.3 ±0.4	4.0±0.4	126±8	BD
D-Val	8.2±0.4	8.0±0.5	1.47±0.13	0.75±0.07	LOQ	3.23±0.05	0.97±0.04	BD
L-Val	310±20	290±20	51±5	30.7±1.2	5.65±0.84	179±17	39±4	BB
D, L-Pro	120±12	134 ±3	27.7±1.6	9.2±0.8	1.42±0.14	37.6±1.1	24.9±1.1	BB
D-Glu	56±4	76±4	20±2	10±2	BD	BD	24±2	BD
L-Glu	620±40	620±40	174±8	78±9	LOQ	19.5±1.6	127±8	BD
D-Iso-Leu	5.0±0.3	4.9 ±0.3	0.65±0.05	0.40±0.04	LOQ	2.44±0.12	LOQ	BD
L-Iso-Leu	159±14	159±14	20±2	14.1±0.8	3.7±0.2	96±9	20.7±1.6	BB
D-Leu	7.7±0.5	7.7 ±0.5	0.77±0.06	0.56±0.07	LOQ	3.7±0.3	0.85±0.06	BD
L-Leu	237±16	237 ±17	25±2	19.8±1.1	1.69 ±0.2	113±8	26.1±1.8	BB
D-Phe	7.0±0.5	7.0±0.5	0.44±0.08	0.60±0.08	BD	0.87±0.06	LOQ	BD
L-Phe	141±9	141±9	9.7±0.7	13.8±0.4	1.11±0.08	33±3	14.7±1.2	BD

BD: below detection sensitivity; BB: below blank; LOQ: limit of quantification; n.a.: sample not measured

FIGURE CAPTIONS

FIG. 1*: Location of the study area within the Atacama desert. ASTER global digital elevation model (GDEM) is overlain in rainbow scale over ArcGIS world topographic map (ArcGIS world imagery maps, ESRI/DigitalGlobe). The ASTER GDEM is a product of METI and NASA. Salars and sampling sites are indicated as magenta outlines and white stars respectively. For Sample ID's and the names of their respective salars, see Table 2.*

FIG. **2***: The different collections sites are shown and numbered according to their Location ID (see Table 2). Further information is listed in Tables 1 and 2.*

FIG. 3*: Relative contribution of 9 selected (most dominant) amino acids per investigated sample. ATA01-ATA04: Salar de Laco; ATA05-ATA09: Laguna Tuyajto; ATA10-ATA13: Salar de Aguas Calientes 3; ATA14-ATA16: Salar de Atacama (Peine road). ATA06 was not measured for amino acids and for ATA17 no amino acids were detected.*

FIG. 4*: DNA concentration in the extracts of the analyzed soil samples shown in logarithmic scale. Several samples were shown to have DNA load below our initial detection limit, but all except one (ATA14) revealed the presence of enough genomic material for analysis after PCR amplification.*

FIG. 5*: Observed operational taxonomic units (OTU's) per sequenced soil sample in the rarefied dataset*

FIG. 6*: Stacked barplot depicting the microbial diversity in the soil samples at Phylum level according to the rarefied 16S amplicon sequencing dataset*

FIG. 7*: Principle coordinates analysis (PCoA) plot using weighted unifrac metrics visualizing the beta diversity (how different the samples are in terms of species composition and relative contribution) of the microbial communities at the OTU level present in the samples from the different salars. Individual samples are coloured according to their respective salars*

FIG. 8*: Organic carbon and nitrogen load in the soil samples shown in weight percentages. Nitrogen values are enhanced by a factor 10 in this figure for clarity and visualization. Measurements were performed in duplicates and standard deviations of the mean are shown.*